

*New Hampshire Department of
Environmental Services*

Biomonitoring Program Protocols



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1. Program Overview

Preserving, maintaining, and restoring the physical, chemical and biological integrity of our nation's waters are goals expressed in the Federal Clean Water Act and shared by the New Hampshire Department of Environmental Services. The Department's Biomonitoring Program (the program) assesses the biological health and integrity of aquatic ecosystems throughout the state, focusing on wadeable streams. The results of these assessments are used to establish reference locations for "least disturbed" conditions in the state, and to identify areas that are biologically impaired. Eventually, such information will aide in prioritizing those areas needing management, restoration, or preservation efforts.

The Clean Water Act encourages states to implement a plan to develop numeric biological standards. The Biomonitoring Program is working towards calibrated metrics that will be eventually incorporated into the State of New Hampshire Surface Water Quality Regulations. Currently, there is a narrative biological standard, which reads as follows:

Env-Ws 1703.19

Biological and Aquatic Community Integrity.

- (a) The surface waters shall support and maintain a balanced, integrated, and adaptive community of organisms having species composition, diversity, and functional organization comparable to that of similar natural habitats of a region.
- (b) Differences from naturally occurring conditions shall be limited to non-detrimental differences in community structure and function.

The primary purpose of this document is to outline methods the Biomonitoring Program uses to further define, and eventually enumerate expressions in the narrative statement. For many methods, the program adheres closely to the US Environmental Protection Agency's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers. Monitoring activities taking place at most sites include:

- Collection and identification of aquatic macroinvertebrates
- Collection and identification of the resident fish community
- Assessment of riparian and in-stream habitats
- Physical and chemical measurements for assessing water quality

Since its beginnings in 1995, the program has continually expanded its assessment capabilities. The primary focus has been and remains on wadeable streams, with numerous special projects in other habitats. It has been determined that consistency in data collection is vital when analyzing and comparing community metrics. Therefore, even though the program continues to incorporate new methodologies, a single established method is used when generating data for the purposes of biocriteria development. For macroinvertebrates, the established method is rock baskets, placed in the stream for 8 weeks. Kick-net sampling is done on specific projects where rapid turn around is warranted. Some stations have also been sampled using kick-nets when rock baskets were vandalized or washed away. Fish are collected by a single-pass method for 150

meters. The protocols for these activities have remained fairly consistent since the program began.

The Biomonitoring Program aims to gain knowledge of and expertise in sampling other types of habitats, such as deep-water rivers, wetlands, and impounded areas. That is why we have developed and tested the D-net sweep protocol, primarily for slower backwaters and wetlands, and the Hester-Dendy multi-plate sampling device for deep-water benthic assessments.

It is important to us that all methods be thoroughly documented, especially those that will be used over the long-term for biocriteria development. This document provides detailed descriptions of all data collection and processing used by the program. We encourage other groups interested in comparable data to follow these protocols to the degree warranted by their specific project goals.

2. Site Selection

The state of New Hampshire has great variability in stream types, from mountainous, high-gradient systems to lowland coastal systems. In order to develop biocriteria that apply to all types of streams, we endeavor to sample all types with equal effort. In this section we explain how sites are broadly chosen and then how in-stream reaches are selected. The process of site selection is on going for the program, and others in the Department often assist by making suggestions of potential sites based on something they have observed in their own field operations. The selection of reaches for biocriteria development differs from the selection of reaches for targeted, site-specific investigations. When appropriate, upstream-downstream bracketing will be done for investigations of a known or presumed impairment.

2.1 Statewide Site Selection

The program is currently using a discretionary process for selecting sites. Generally, only streams of third order or higher are considered to avoid ephemeral conditions. The initial emphasis of the program was to collect data from least impacted conditions. Impact was determined by using GIS coverages and avoiding RCRA and CERCLA sites, NPDES outfalls, urban areas, flow controls, or other discernable indications of impact. In 1999, in order to begin covering a range of disturbance, the above-mentioned impacts were sought for biomonitoring stations. The program still seeks impacted sites, utilizing anecdotal reports from the Hazardous Waste Remediation Bureau, from Department people involved in enforcement, and from the Water Quality Section's 303(d) list. Another factor that weighs in more heavily at this time is targeting locations that fill geographic data gaps statewide. As our total coverage increases, geographic areas that have been difficult to access become more apparent. For example, urban streams are often culverted, or disappear underground. It has been difficult to locate 150-meter reaches in heavily urbanized areas. There are also areas within the White Mountain National Forest that have been difficult to sample, due to lack of convenient access.



In 2002-2003 the program will be participating in a region-wide probabilistic stratified random sampling study being conducted by Region I Environmental Protection Agency. It is thought that this type of computer-generated sample set can be extrapolated to conditions statewide with a high level of confidence. This probabilistic, random sample approach may be adopted as the site selection process for the program in the future.

2.2 In-Stream Reach Selection

Proper selection of in-stream sampling locations is a key component for implementation of a successful survey. Stream flow velocities, canopy cover, substrate type, water temperature, and water depth should be considered. Inconsistencies in data due to mismatched habitat types cannot be eliminated entirely, but good, comparative evaluations require as much constancy in site selection as possible.

Typically, the key habitat type for monitoring lotic systems is the riffle area, where the faunistically richest habitat is most likely to be encountered, especially for macroinvertebrates. However, in the diversity of New Hampshire streams, the riffle habitat is not always present. Many high gradient streams are boulder-dominated or “bony”. The low gradient coastal streams may have softer substrates bordered by macrophytes. The program uses rock baskets expressly for the purpose of eliminating some of the in-stream habitat variability. The placement of the baskets is critical however, since they must be in areas where water will continually move over and through the rocks in the baskets. They also need to be located in an area where depth will be adequate to keep the all baskets covered for 8 weeks. Considering the goals of both macroinvertebrates and fish, the stream reach needs to be wadeable and contain a suitable location for rock baskets. Typically, the riffle/run sequence and/or and mixture of pools and glides will suit all of these requirements.

One final consideration in selecting the location for the surveys is access. The Biomonitoring crew needs to be able to get gear to the stream multiple times over the sampling season. The best option for this is to locate a bridge crossing and look for suitable sampling habitat upstream. This minimizes traversing private lands and can save valuable field time.

2.3 Pre-Season Preparation

Typically, prior to field season, a large number of sites are selected - more than is reasonable for a field season. Traveling routes are planned for reconnaissance, and many potential stations are eliminated once they have been visited and deemed unsuitable. Once the number and locations of the chosen sites are confirmed, **Biomonitoring Station Information Sheets** (Appendix A-1) are created. These sheets become part of a site packet with all of the other needed field sheets. **Biomonitoring Station Information Sheets** include GPS readings, NH Atlas and Gazetteer reference pages, and narrative directions.

3. Macroinvertebrate Collection

One of the advantages of using macroinvertebrates in assessing the health of aquatic ecosystems is their ease of collection. Benthic organisms are typically abundant in most streams and can be identified by experienced biologists. Minimal equipment is necessary for collection, and the process can provide a cost effective approach to assessing habitat and water quality in New Hampshire streams and rivers.

3.1 General Considerations for Macroinvertebrate Sampling

Method of collection for macroinvertebrates depends primarily upon the physical characteristics of the water body. Is it a deep-water habitat? Is it a stagnant backwater? Is there three feet of silt and mud in the stream channel? Is there a tremendous current? These are some of the observable factors that will dictate the best method for gathering organisms inhabiting the area. Also, some methods provide only qualitative information, such as the types of taxa present. Other methods are semi-quantitative, providing relational taxonomic information. The most quantitative methods allow for biomass estimates on a per-unit basis. Depending on the objectives of the survey, all factors need to be carefully considered in order to determine if the proposed methodology will meet the data quality objectives.

Sampling techniques are different for wadeable and non-wadeable streams. Our current protocols include **rock baskets**, **multi-plate samplers**, **kicknetting**, **D-net sweeps**, and **sediment grabs**. Each method has advantages and disadvantages. For example, rock baskets and Hester-Dendy multi-plate samplers supply semi-quantitative data. These types of samplers allow for surveys in study areas that have limited substrate for colonization, such as bedrock or clay bottoms. Both of these devices can also be used in deep, non-wadeable or high velocity flow areas where net-collection is impossible. However, the artificial substrates are more labor intensive and require additional logistical considerations. They require two trips to the site, and there is a possibility of sample loss due to vandalism or unforeseen disturbances. On the other hand, net-collected samples require only one site visit, and vandalism and unforeseen losses are not issues.

Both kicknetting and artificial substrates provide reliable and consistent data to biologists. Questions of whether the two methods are comparable to one another are debated in stream ecology. Can artificial substrate data be compared to kick net data across several locations? In order to remove this uncertainty, the biomonitoring program tries to use rock baskets at all “routine” sites if feasible. We often are involved in special projects with different hydrologic characteristics, requiring other sample methods.

3.1.1. Qualitative, Semi-Quantitative or Quantitative?

Ecological data is often categorized as **qualitative**, **semi-quantitative**, or **quantitative**. Generally, qualitative data measures the presence (or absence) of individual taxa. This results in a list of taxa from the sampling effort. From this, approximate relative abundance among taxa can be calculated. This type of data is suitable for narrative summaries and might best be employed as an initial screening. Quantitative data enumerates organisms in each taxon, and also measures the assessment area. This type of data allows for detailed



statistical analysis and expressions of biomass per unit area. The Biomonitoring Program considers the macroinvertebrate methods used by the program to be semi-quantitative. The organisms are enumerated from an area that is always contained and defined but not measured precisely. For example, the rock basket units are self-contained and always of the same spatial dimensions. They provide all organisms an equal opportunity for colonization, which effectively eliminates sampler bias. This allows solid comparisons among taxa, beyond approximate relative abundance. However, we do not measure the actual surface area of the rocks within each basket. For this reason, we could not make statements about the organisms on a per-unit basis.

3.1.2 Sampling Season for Macroinvertebrates

Although macroinvertebrate sampling can be done at any time of the year, the program targets mid summer to early fall. This is a period when most macroinvertebrates are in the later instars and are large enough to be captured and identified with the greatest level of confidence. Late July through September, prior to leaf drop, provides a fairly stabilized food source most likely to support a balanced indigenous community. Also, this time frame is usually the most representative of sustained annual low flows and reflects (with some exceptions) hydrologic conditions generally most stressful to instream biota. This provides a “worst case scenario” for the resident community.

If surveys are conducted in subsequent years for the purpose of trend monitoring, then sampling should be conducted within the same time periods from year to year, with the assumption that flows and degree days will be relatively constant over the long term. Monitoring may be conducted at anytime of year for point-source impact assessments when upstream/downstream stations are to be used.

3.2 Macroinvertebrate Collection in Wadeable Streams and Wetlands

Wadeable streams are defined as lotic waterbodies permitting the passage by foot. This definition obviously requires seasonal and individual interpretation. Factors that make a stream non-wadeable include depth, difficult substrate (muck), and high velocity. Rock basket artificial substrates and “kick” sampling are the two predominant methods utilized by the program for sampling lotic benthic macroinvertebrate communities in wadeable streams.

The colonization of rock baskets by macroinvertebrates is usually more selective towards the scraper and collector-filterer communities, and may differ from the resident community. In such cases, artificial substrates are more representative of the potential for colonization than of the resident community. These factors should be considered in the survey planning stages

Kick sampling is applied when qualitative or semi-quantitative assessments are desired, for resident macroinvertebrate community determinations, and for rapid biological assessment purposes. The approach is limited to wadeable stream reaches with flow velocity ranges predominantly within 0.5 to 2.5 feet/second.

The D-net sweep is the third macroinvertebrate collection technique used by NHDES in wadeable streams. It is similar to kick sampling as it provides qualitative or semi-quantitative assessments. The program uses this protocol in such habitats as wooded floodplain areas or unchannelized streams without a distinct riparian corridor. Typically, these streams lack riffles and have a flow less than 0.5 feet/second.

3.2.1 Rock Baskets

Rock baskets are comprised of regionally indigenous bank run gravel ranging in size from 1.5 - 3.0 inches in diameter and are housed in a 6.5 inch diameter cylindrical plastic coated wire basket 11 inches in length. The bottom of the baskets is hinged and once filled with rock, the hinged door is secured with three plastic cable ties. The mesh opening is 1 square inch (Figure 3-1). Baskets are placed in stream habitats at depths that cover the artificial substrate by at least 5 inches. Each biomonitoring station uses three baskets that are anchored to the streambed by sinking $\frac{1}{2}$ inch steel reinforcing rod and then attaching the baskets downstream in an array pattern with a loop of nylon coated steel cable. In an effort to deter removal by vandals, all three basket cables are secured to the rod with a plastic cable-tie.



Figure 3-1. Rock baskets in an array of 3, secured to a "rebar" with nylon coated steel cable.

Substrates should be left undisturbed at the site for a period of eight weeks in order for adequate colonization to take place. The baskets should be retrieved within a couple days of the allotted time frame.

Rock basket retrieval is done by approaching the substrate from downstream and placing a 3-gallon sieve bucket of 600- μ m pore size against the stream bottom just downstream of the substrate. Locate and snip the plastic cable-tie that secures the three baskets to the rod. Debris/algae clinging to the rock basket should be gently removed and discarded and then the top basket quickly lifted inside the bucket. Once the rock basket is placed into the bucket and removed from the water, both the bucket and rock basket are transported streamside. The sample retrieval protocol for rock baskets should proceed as follows:

1. Using a knife or nail clippers, cut the plastic tie wraps securing the basket's hinged door and empty the contents of the basket into the sieve bucket
2. Add 3-4 gallons of water to a 5-gallon pail and place the empty rock basket into the pail. Using a soft bristle brush, gently scrape all organisms attached to the basket into the pail. Empty pail contents into sieve bucket and carefully rinse.
3. Add 2-3 gallons of water to the 5-gallon pail and nest the sieve bucket containing the rocks into the pail. The rocks should now be covered with water. Using a soft bristle brush, lift rocks from the sieve bucket and gently brush organisms and detritus from the substrate (Figure 3-2). After each rock has been scrubbed, return it to the basket cage.



4. When all organisms and detritus have been removed from the rocks, lift the sieve bucket from the 5-gallon pail to remove the water. Large substrate materials and detritus that have been collected should be cleaned of organisms and returned to the stream.
5. Transfer remaining contents of sieve bucket into a one quart wide mouthed jar and preserve with 1/3 water and 2/3 ethanol.
6. The sample jar is labeled using an indelible marker with the appropriate information of date, replicate number, and site number.
7. Repeat steps A-F for each of the remaining replicates.
8. Re-secure the hinged door of the filled rock basket with three plastic cable ties. Sampler substrate material should be thoroughly cleaned and allowed to dry for a sufficient time for complete desiccation before reuse.

3.2.2 Kicknets

The technique employed for kicknetting is to disturb or “kick” the stream bottom while placing a net immediately downstream and collecting those organisms which have become dislodged from the substrate. Bottom substrate should be comprised of coarser materials such as rocks, gravels, and sands, preferably located in riffle areas. The in-stream sampling areas should be located in the middle two-thirds portion of the channel to avoid variances from riparian habitat communities and should have consistent substrate material and similar depths and flow velocities. Areas which are prone to eddy currents from large substrate materials (i.e. Boulders, logs) should be avoided.



Figure 3-3. Kicknetting procedure.

Disturbance of substrate materials upstream prior to and during sampling should be avoided. However, if



Figure 3-4. Quadrat, 1/5 square meter for quantitative kicknet

electrofishing and kick netting are to take place at the same site, then kick-netting should be implemented after electrofishing is completed to avoid disturbing the resident fish. It is assumed that fish have greater mobility and will more likely vacate the area. Standard equipment is an 18" rectangular frame net with a 600-micron mesh size, held against the stream bottom to collect the organisms as they are dislodged from the substrate. Some discretion is used to avoid large or irregular substrate when placing the net on the stream bottom. The net must make solid with the substrate. A quadrat, common in terrestrial sampling, is used when a quantified area is needed (Figure 3-4). The sampling protocol for rectangular frame net samples is as follows:

1. Looking upstream, randomly toss a one-fifth square meter quadrat (Figure 3-4) into the riffle area.
2. Place the 600-µm kicknet on the bottom of the stream directly downstream of the quadrat. Disturb the area within the frame by rubbing stones and stirring up embedded sand and gravel with hands and feet for one minute (Figure 3-3).

3. While remaining in the middle two-thirds portion of the streambed, move upstream and randomly toss the quadrat once again. Continue this procedure until a total of five one-minute collections have been completed, representing one sample collection of a randomly selected one-square meter area.
4. Empty the contents of the net into a white enamel pan and add any organisms that remain attached to the net. This facilitates stream-side sorting and only the organisms, teased from the debris, are placed in a sample container. Alternatively, the contents of the net can be directly transferred into sample jar:
5. Preserve jar with 1/3 water and 2/3 ethanol.
6. Place a label on the jar using an indelible marker with the following information: date, replicate number, and the site number.
7. Proceed upstream of the first sample collection point and return to Step 1, repeating the process for replicate samples.

3.2.3 D-net Sweep

The methods used for the D-net sweep protocol have been adapted from US EPA Region 3 Field and Laboratory Methods for Macroinvertebrate and Habitat Assessment of Low Gradient, Nontidal Streams, Mid-Atlantic Coastal Streams Workgroup. They have been minimally revised to suit the needs of NHDES.

Field collection will involve the use of the 1-foot wide D-frame dip net with a mesh size of 650 μm , having heavy canvas sides to protect the mesh from tearing when jabbing in snags and woody debris. Macroinvertebrate collection consists of repeatedly jabbing the D-net in productive habitats, with a single jab consisting of aggressively thrusting the net into the target habitat for a distance of approximately 1 meter (roughly the distance the net can be swept while standing in one place). The resulting level of effort represents a standardized sample area of approximately 2.7 square meters. The locations of the jabs are selected according to the proportion of these habitat categories present in the assessment area.

Habitat categories:
Snags and overhanging woody brush
Root mats
Muck/Silt
Vegetation/Macrophytes
Hard banks

For example, if the assessment area has three stable habitats present, then 3 jabs are located within each representative habitat type. This standardizes the selection of habitats to be sampled.

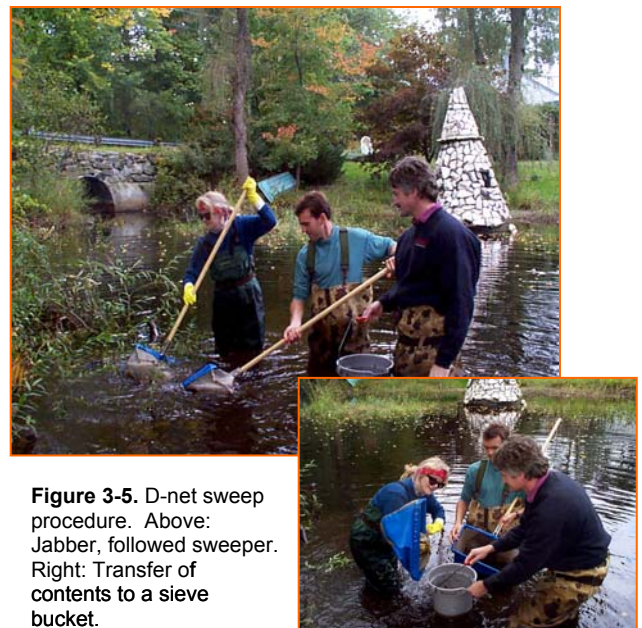


Figure 3-5. D-net sweep procedure. Above: Jabber, followed sweeper. Right: Transfer of contents to a sieve bucket.

In areas with soft substrates where wading is not feasible, sampling will be conducted by wading along the edge of the stream or by standing on the bank out of the stream

channel. Sampling of the channel bottom (sand, muck, and detritus) should be avoided since these habitats are relatively unproductive. Productive habitats along muddy bottoms can be effectively sampled by bumping the net along the bottom rather than by dragging the net through the substrate.

Sampling with the D-net Sweep method should proceed as follows:

1. Locate productive habitats to sample and determine, proportionally, the sampling effort required for each habitat.
2. Jab the D-net in the selected habitat. Follow jab by 2-3 sweeps of the same area to collect dislodged organisms (Figure 3-5).
3. Move upstream to next habitat, avoiding recently suspended sediments. Repeat step 2.
4. Continue until a total of 9 jabs have been performed.
5. Follow steps 4 – 6 of **3.2.2 Kicknets** sampling protocol for macroinvertebrate processing.

NOTE: It may be desirable to retain jabs in different habitats in individual containers, if this suits the study design.

3.3 Macroinvertebrate Collection in Non-Wadeable Streams

Non-wadeable streams present physical barriers to macroinvertebrate collection and require special sampling considerations. Hester-Dendy multi-plate samplers and sediment grabs are the two methods the Biomonitoring Program employs for macroinvertebrate collection in non-wadeable streams. Hester-Dendy samplers could be used quantitatively if the total surface area is measured. Sediment grabs also provide quantitative data as they sample a defined volume of substrate. The method used will be determined by study objectives.

3.3.1 Hester-Dendy Multi-Plate Samplers



Hester-Dendy multi-plate samplers are highly portable and have a diversity of applications in both wadeable and non-wadeable streams. The Biomonitoring uses them primarily for non-wadeable collections. The devices used by the program were designed using US Geological Survey Methods for the Collection and Analysis of Aquatic Biological and Microbiological Samples. The device suspends three multi-plate stacks from a single top bar.

The bar is tethered to floats and also to two anchors. The ropes on the anchors are adjusted to hold the entire device in place with a limited amount of play. The three samplers themselves should be adjusted to rest just above the sediment-water interface. (Figure 3-6), A description of the dimensions of the Hester-Dendy multi-plate sampler as well as assembly materials and

Figure 3-6. Hester-Dendy multi-plate device. Inset: individual Hester-Dendy multi-plate stack.

instructions for the complete device can be found in Appendix C-1.

Hester-Dendy units are set perpendicular to flow so that the plates will not interfere with each other and to ensure uniformity of conditions for all three stacks. Optimal macroinvertebrate colonization period for the Hester-Dendy units is 6-8 weeks. The following method places all three multi-plate stacks into one sample. Slight adjustments in the retrieval method would allow each sampler to be kept separate.

If conditions are truly non-wadeable, setting and retrieving these samplers is best accomplished by canoe. It can also be done by wading or swimming, assuming conditions are safe.

Setting Devices

1. Deploy Hester-Dendy device perpendicular to flow by proper placement of brick anchors. Adjust the anchor rope so that there is a slight drift downstream.
2. Set depths of individual multi-plate units by raising or lowering the threaded rods. The threaded rods and the length of anchor rope may need a series of adjustments to make the unit stable, and to keep the stacks just above the sediment.
3. Check apparatus for its ability to remain stable and perpendicular to flow in the current, and inspect unit for retention of proper depth placement.

Retrieving Devices

1. Place dipnet on substrate immediately downstream of units.
2. Lift units just enough to quickly place into dipnet. Immediately bring complete unit to the water's surface.
3. Detach each Hester-Dendy multi-plate sampler, disassemble, and place spacers and plates in a sieve bucket.
4. Gently scrub all pieces in sieve bucket and re-assemble Hester-Dendy units.
5. Follow steps 4 – 6 of section **3.2.2 Kicknets** sampling protocol for macroinvertebrate processing.

Sampler substrate plates should be thoroughly cleaned and allowed to dry for a sufficient time for complete desiccation before re-use.

3.3.2 Sediment Grabs

Sediment grabs can be performed by either an Ekman grab or a Ponar grab (Figure 3-7). The Ekman grab is lighter and is used for sampling soft substrates, whereas the Ponar grab is used when sampling from compact substrates. Large rocks, sticks, or other debris should be avoided at the sample collection site as they may interfere with the operation of the hinged doors on each of the devices. The grabs are typically deployed by boat, lowered to the bottom, then tripped with a

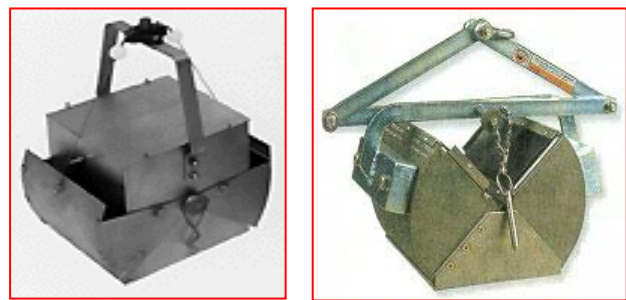


Figure 3-7. Ekman grab (left) and Petite Ponar grab (right)



messenger, and brought back up to the boat for processing. Sampling methods for both devices are the same and should follow these steps:

1. Tie one end of messenger rope to the Sediment grab sampler. Secure sampler by tying other end of rope to the boat.
2. Determine depth of water column. If depth cannot be pre-determined, use grab sampler to find the depth. Then, before sampling, lift sampler off of substrate and move to undisturbed area.
3. Immediately after sampler touches the substrate, trip the Sediment grab sampler by sending messenger down the line.
4. Regain sampler by even flowed retrieval, taking care to avoid sediment loss.
5. Follow steps 4 – 6 of section **3.2.2 Kicknets** sampling protocol for macroinvertebrate processing. Fill wide-mouth preserving jar no more than $\frac{1}{2}$ full with sample.
6. Thoroughly rinse Sediment grab sampler before re-use, with subsequent visual inspection to ensure that no specimens remain on the sampler.
7. Repeat steps 2 - 6 as needed.

4. Fish Collection

Monitoring the resident fish community can provide important insight to assessing the overall habitat and biological integrity of a particular water body. The fish community represents the apex of the aquatic food web, and provides an important endpoint for ecological assessment efforts. The comparatively long life span of fish provides the opportunity to observe how chronic or episodic pollutant events effect the individual as well as the entire fish community over varying temporal and spatial scales. Feeding preferences among various fish species provide opportunities to observe changes in the community over time as food resources become contaminated or depleted due to non-natural causes. Shifts in community composition occur due to altering food resources, changes in habitat structure and availability, and changes in water chemistry. Noticeable deviations from expected population and trophic levels can provide early warning signs to aquatic habitat degradation and can catalyze efforts to mitigate problems and issues within the water body.

When observations of individual fish and/or its community are used in conjunction with the relatively sessile macroinvertebrate community, the result is a comprehensive and robust assessment for determining the aquatic health of the water body. Fish are relatively long lived, cover a broader spatial range, are sensitive to a wide range of physical and chemical pollutant effects, and are readily captured and identified. For these reasons, the fish community is utilized and recognized as an important component to the biological monitoring programs efforts.

The Biomonitoring Program fish assessment protocol is a fairly intensive effort and is adapted from the United States Environmental Protection Agency's Rapid Bioassessment Protocols. The methodology is designed for wadeable streams and rivers, and uses backpack electrofishing equipment.

4.1 General Considerations for Fish Collection



4.1.1 Sampling Season

Surveys are conducted at a time when fish populations are most stable and variability is not induced by the seasonal migration. Extremes in flow conditions can result in non-seasonal migration of certain species seeking less stressful habitat conditions. Therefore, sampling should also be avoided during brief periods of natural high flows, such as following severe thunderstorms.

Fish surveys are conducted by the biological monitoring program from the beginning of June through the end of September, but may be extended into or through October if need warrants. This sampling time frame represents a stable fish assemblage, when fish tend to remain in a particular localized area and is most likely to include the full range of resident species. If a survey is requested or required later in the fall, New Hampshire Department of Fish and Game is contacted for advice on avoiding fall spawning species.

4.1.2 Fish Collection Permits

Prior to any fish surveys, a scientific collection permit must be obtained from the New Hampshire Fish and Game Department (NHF&G). A written letter to the commissioner of the NHF&G from the head of the biomonitoring program should be sent out well ahead of the scheduled sampling period, three months prior to sampling is suggested as a minimum. The NHF&G requests a report be submitted to their office summarizing the results of the survey efforts. This request can be fulfilled by sending out a standardized data report of the sampling survey stations from the biomonitoring database.

4.1.3 Training and Safety for Electrofishing

Electrofishing equipment can be hazardous if not operated competently by trained individuals. It is the policy of the biological monitoring program that any individual operating electrofishing equipment be trained and certified through a training course offered by the US Fish and Wildlife Service. In addition, all individuals on the shock crew must have current CPR training. Any new field personnel are required to attend a field orientation on the proper use and procedures for electrofishing with the biological monitoring program, and are required to read the **Electrofishing Principles Manual** and **Electrofishing Safety Manual** provided by the manufacturer (Smith-Root, Inc.). A synopsis of the training materials is provided in Appendix D-1. At the completion of these activities and readings field personnel are required to sign a waiver (Appendix D-2) verifying that they have read and fully understand the hazards involved and the required safety protocols prior to any actual field survey work.

In addition to the safety measures outlined in Appendix D-1, the field crew should discuss a process to ensure that operation of the shock unit will be immediately discontinued in the event of a fall or stumble. A short word such as “out!” will alert the operator to cease fishing, as a hazardous condition exists.

All field crewmembers are required to wear protective equipment, including waders and rubber gloves (Figure 4-1). If a member does not have these basic items, they will not be

allowed to be in the water during the shocking. Wading belts and life jackets are available if a crew member chooses to use them.

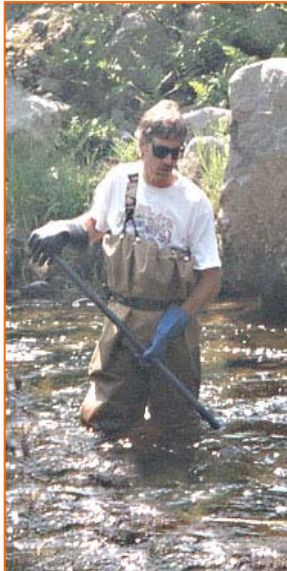


Figure 4-1. Proper electrofishing gear includes chest waders, high voltage lineman's gloves, polarized glasses, and a wading belt.

In order to ensure safety and efficiency in fish sampling efforts as well as equipment longevity, equipment must be maintained appropriately. Proper care will result in less problems encountered in the field, less down time from unnecessary repairs, better performance from the equipment, and less hazard to the users. A field survey checklist can be found in Appendix C-2 and should be completed prior to leaving the office on any field surveys.

4.2 Electroshocking

Accurate representation of the fish species present in the water body and their relative numbers is imperative in order to effectively use this community for assessing water quality. It is also desirable to establish a standardized sampling reach for comparative purposes. Estimating a minimum and maximum sampling distance within which at least 95% of the species were represented derived a sampling reach of 150 meters. Figure 4-2 demonstrates the relationship between number of species and sampling distance: as sampling distance increases, the maximum

number of species captured is approached. It is assumed that where that number plateaus, that sampling distance will capture 95% of the species present. The distance necessary to capture the majority of the species present is a function of stream order, geomorphologic characteristics, gradient, and other physical factors. For wadeable streams in New Hampshire, a minimum distance of 150 meters will be used. This distance has been established as a reasonable limit to prevent unnecessary over sampling, while optimizing efficiency and representation of the resident species.

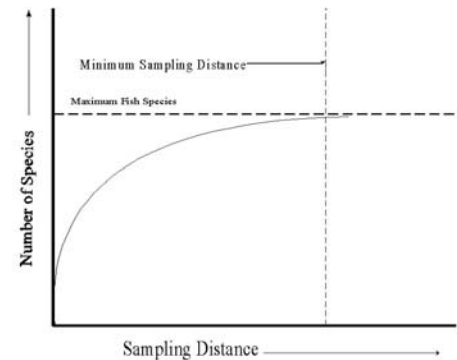


Figure 4-2. Relationship between the number of species vs. sampling distance

A single pass through the 150-meter reach will be conducted working upstream. Sampling in an upstream direction eliminates problems of turbidity from the survey crew and facilitates netting of fish as they drift towards the crew. Different fish species will inhabit a variety of micro habitat types and effort is made to shock all niches present in the stream reach.

The field sampling crew is comprised of one shocker, a minimum of two netters, and one person to carry a 5-gallon, aerated bucket and a spool containing 150 meters of sturdy line. The spool line should be tied off at stream bank at the starting point. Netted fish should be immediately transferred to the 5 gallon bucket and not retained in the net. This will eliminate “double dipping” the already shocked fish.

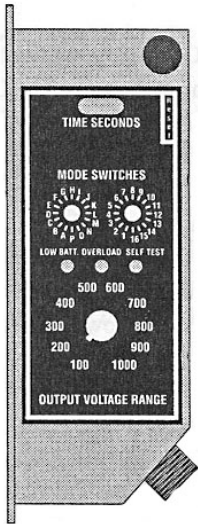


Figure 4-3. Side panel of electroshock unit, with dial settings for voltage and modes, and shocking time display.

Numbers, species and shock time in seconds are recorded to determine the catch per unit of effort (CPUE), providing another level of standardization for making comparisons between sites. The programmed waveform for the pass should also be noted (i.e. Voltage setting, pulse width/pulse frequency). Initial settings should be 60Hz at 6ms which is the I5 mode setting (Figure 4-3). Initial chemical data including pH, temperature, dissolved oxygen and conductivity are made on the Biomonitoring Site Information Sheet (Appendix A-1) before electrofishing. Conductivity levels as well as the targeted species or size class to be captured, will dictate the waveform settings of the backpack shocker. Where macroinvertebrate monitoring is scheduled to take place in conjunction with fish community assessments, the kicks sites or rock basket placement should be within close proximity to the fish sampling reach.

4.3 Fish Sample Processing

Processing of the survey sample is conducted after the single pass. Numbers and types of species are recorded on the standardized Fish Data Collection Sheets (Appendix A-2). Fish less than 25mm in length are not included in the tabulation. If these small fish are especially abundant and can be identified, then an appropriate note will be made on the field sheet. External anomalies will be noted on the field sheets as well as an estimation of the number of fish exhibiting the anomaly. All fish will be retained in the aerated 5 gallon bucket until tabulated, and then released back into the water body. Non-identifiable fish may be retained for identification back in the laboratory.

5. Habitat Assessment

Surrounding topographical features and instream physical characteristics can govern the composition and quality of the resident aquatic community. Although the Biomonitoring Program limits most assessment efforts to wadeable streams, enough habitat variation exists within this stream type to require the use of accurate habitat assessment data when interpreting biomonitoring results.

5.1 Stream Flow

The Biomonitoring Program measures stream flow during every visit to each station. There are many advantages to having this supportive data when analyzing macroinvertebrate and fish data. Existing flow conditions will have bearing on electrofishing success, generally dictating how many escapes or “flow bys”. Flow data also provides a moment-in-time account of conditions that are available for macroinvertebrates. Although it is unknown how flows fluctuate between rock basket deployment and retrieval, we at least have a quantitative measurement at the time of these events. Flow measurements at each visit also provide an understanding the watershed.



For example, if there has been no rainfall in the area for a month and yet the flow remains constant, we can make some judgments about the predictability of a stream.

Stream flow data is obtained using a Marsh-McBirney flow meter, displaying the flow in feet per second (F/S). This instrument has a bulb suspended in the water column that has a potentiometric sensor on its face. In order to be most effective, the bulb must be placed directly into stream flow. The program uses the 6/10ths depth, which is the standard depth and considered most representative of the vertical column. When selecting a cross-section for flow measurements, care must be taken to avoid areas that are not representative. Stream areas to avoid include non-uniform vertical flow, non-uniform channel substrate, tree down falls, large rocks, and low flow in wide cross-sections. Understandably, field conditions are typically less than optimal and best professional judgment must be used when choosing these cross-sections.

Cross-sectional stream width determines the number of intervals to be measured. Although the program uses no specific formula to derive the minimum number of intervals, cross-sectional widths under 20 feet have interval widths of 0.4 to 0.6 feet. Cross-sectional widths over 20 feet typically have a minimum 1 to 2 foot wide interval. Depths are recorded at the beginning, middle, and end of each interval. Flow is recorded in the middle of each interval. Final calculation in cubic feet per second (CFS) is performed using an MsExcel spreadsheet. Stream flow measurements procedure is as follows:

1. Locate optimal section of stream (see considerations above).
2. Tightly stretch measuring tape across the stream and secure with a stick or tie off to shore structure.
3. Record total stream width and determine the appropriate number of intervals.
4. Record water depth at the beginning, middle, and end (Bank #1) of every interval until Bank #2 is reached. The final interval may not be as wide as the rest.
5. Record flow in F/S in the middle of each interval. Flow meter bulb should suspend at roughly one third water depth and point in the upstream direction.

5.2 *Habitat Assessment Sheets*

The Biomonitoring Program has incorporated EPA's habitat assessment protocol, which focuses on the physical characteristics of a particular site, encompassing a 150-meter reach. Low and high gradient **Habitat Assessment Field Data Sheets** are found in Appendix A-4. The condition of the habitat at a particular biological monitoring station is evaluated utilizing ten different parameters. Each of these parameters is assigned a score based on visual observations of a team of at least four Biologists. Most habitat parameters are scored from 1 to 20. Although, three of these parameters are broken into two parts, left and right bank, and scored from by bank from 1 to 10.

Each biologist participating in the monitoring study conducts their own habitat assessment, typically after biological and chemical parameter collections, and then opens discussion until agreement is reached on the overall condition of the habitat. In this manner, a semi-quantitative and standardized approach to assessing the habitat is reached through consensual best professional judgment. By looking at these individual habitat assessment parameters, one can obtain important information regarding community structure and

health. Interpretation of habitat assessment data can also be accomplished by summing the ten habitat parameter scores for an overall assessment value: 161-200 - optimal, 101-160 - suboptimal, 51-100 - marginal, ≤ 50 - poor.

A description of each of the ten habitat characteristics evaluated at a site can be found on the **Habitat Assessment Field Data Sheets (Appendix 4.1 - 4.4) and further described in EPA Rapid Bioassessment Protocols.

5.3 Additional Station Information

Section 2.3 introduces the **Biomonitoring Station Information Sheets** that contain GPS readings, NH Atlas and Gazetteer reference pages, and narrative directions to the site. Additional information to be recorded on this sheet includes average stream width and depth, a photo of the site, a free-hand planar view drawing, and estimated canopy cover. Special projects may warrant additional parameters as dictated by the individual studies.

6. On-Site Chemical Analysis

Routine on-site chemical analysis is performed using a hand-held Multi-Parameter Unit that measures conductivity, dissolved oxygen, pH, and temperature. These four parameters are measured during each to each site. Not including the initial reconnaissance visit, each station is visited three times for the major biological community sampling: fishing, rock basket deployment, and rock basket retrieval. Additional chemical parameters may be warranted in special projects. The selected parameters provide fundamental water quality information. Probe specifications are found in Table 6-1.

Sensor Specifications for Multi-Parameter Unit

Parameter	Sensor Type	Range	Accuracy	Resolution
pH	Glass Combination Electrode	0 - 14 units	+/- 0.2 units	0.01 Units
Conductivity	4 Electrode Cell with Auto ranging	0 to 100 mS/cm	+/- 0.5% of reading + 0.001 mS/cm	0.001 mS/cm to 0.1 mS/cm (range dependent)
Dissolved Oxygen	Rapid Pulse - Clark type, polarographic	0 to 50 mg/L	0 to 14.6 mg/L, +/- 0.2 mg/L	0.01 mg/L
Temperature	Thermistor	-5 to 45°C	+/- 0.15°C	0.011% air saturation
Depth	Stainless Steel Strain Gauge	0 to 9 meters	+/- 0.02 meters	0.001 meters

Table 6-1. Sensor specifications including range, accuracy, and resolution for the five parameters measured by the Multi-Parameter Unit. All parameters, excluding depth, are recorded during each site visit.

6.1 Hydrolab Chemistry

Conductivity Conductivity measurements can be used as an indicator of soluble ions (dissolved salts) and nutrient enrichment in the water column. Major possible sources of these include chlorides, nitrates, sulfates, phosphates, sodium, magnesium, calcium, iron, and aluminum. The average conductivity for New Hampshire ranges between 40-70 ohms/cm. This measurement is required prior to electrofishing, in order to know the most effective settings for shocking.

Hydrolab Calibration Procedures for Conductivity

1. Rinse sensors several times with DI water.
2. Rinse sensors twice with specific conductance standard.
3. Screw on calibration cup and point sensors upward.
4. Pour in standard to within 1 centimeter from top of cup making sure there are no air bubbles in the cell block.
5. When specific conductance readings stabilize select CALIBRATE SpC/S from the calibration menu.
6. Type in the calibration standard value ($\mu\text{S}/\text{cm}$ or mS/cm) and press ENTER.

Dissolved oxygen Maintaining suitable dissolved oxygen (DO) levels is crucial to the survival of many aquatic species. Low levels of DO can stress organisms and interfere with growth and reproduction, and very low levels can result in fish kills. The DO criteria for class B waters, in New Hampshire is a daily average of at least 75% of saturation with the minimum value being no less than 5.0 mg/L, unless naturally occurring.

Hydrolab Calibration Procedures for Dissolved Oxygen

1. Inspect D.O. probe for air bubbles or damaged membrane. If either occurs, repair according to manufacturer suggestions.
2. With sensors pointed upwards, fill bottom-less calibration cup with de-ionized or tap water to a level just below the D.O. membrane. Wipe membrane free of water droplets by use of a Kim wipe. Place calibration cup cap on top of calibration cup.
- 3.
4. Select CALIBRATE %S/DO from calibration menu.
5. Wait for reading to stabilize.
6. Press Calibrate. Calibration is complete.

pH The measurement of pH is essential to determine living conditions within an aquatic community. The pH of natural waters ranges from 3.0-12.0. Allowable pH standards for New Hampshire range from 6.5-8.0 and are deemed protective of aquatic life. Values falling outside these ranges are considered violations and are harmful to aquatic life, except where they are naturally occurring.

**Hydrolab Calibration Procedures for pH**

1. Rinse sensors several times with DI water.
2. Rinse sensors and calibration cup twice with pH 7.0 buffer solution.
3. Screw on calibration cup and point sensors upward.
4. Pour in 7.0 buffer solution and wait until pH readings stabilize.
5. Select CALIBRATE pH from the calibration menu and type in the value of the buffer (7.0).
6. Repeat steps 1-5 with pH 4.0 buffer solution to set slope.
7. Field values > 7 can be retained without a qualifier only when, upon return to the laboratory, pH 10 buffer reads within ± 0.05 units.

Temperature Temperature within the aquatic habitat can dictate the abundance and diversity of species found in a particular fluvial system. Any drastic changes in temperature besides seasonal changes can effect resident aquatic life.

**The standards for class B waters in New Hampshire include the narrative which states that “any stream temperature increase associated with the discharge of treated sewage, waste, or cooling water, water diversions, or releases shall not be such as to appreciably interfere with the uses assigned to this class.”

6.2 Additional Chemical or Biological Parameters

Special studies may warrant other chemical or biological measurements. Possible meaningful chemical parameters include Acid Neutralizing Capacity (ANC), *E. coli* bacteria, $\text{NO}_2 + \text{NO}_3$, total dissolved solids and total phosphorus. NHDES Limnology Center or the Chemical Laboratory would perform these additional analysis and samples are analyzed by trained, inter-department personnel following established protocols.

6.3 QA/QC

Hydrolab chemical values are documented on the **Biomonitoring Site Information Sheets**. One replicate sample should be run on every tenth sample, and the calibration being performed on the day of use. Additionally, a laboratory logbook will track daily calibrations and all maintenance procedures documented by date, activity performed, and initialed by the person performing the activity.

7. Macroinvertebrate Identification

7.1 General Considerations

The Biomonitoring staff has experience in identifying macroinvertebrates. However, we feel that we do not have the specialized expertise required to achieve genus or species level identification. All samples intended to be part of the biocriteria development data set will be sent to a taxonomic laboratory. On special projects, in-house staff will sort and identify organisms to family.

Sample Splitting



Depending on basket conditions and seasonal variation, the rocks may be silt-laden or covered with detritus and leaves. In cases where sample volume is far greater than what is reasonable or necessary for a meaningful community assessment, splitting the sample may be warranted. In this case, splitting the sample is a means of reducing the volume of a particular sample by retaining only a portion in order to minimize staff time in processing. Both portions of a split sample may be retained and processed as a QA/QC check. The split sample in this case provides indication of the similarity of the individual components. Split sampling will be done by Biomonitoring staff for all samples exceeding approximately one liter of detrital/organismal volume. Sample splitting is accomplished using a standard No. 30 sieve. The sample is placed onto the sieve, immersed in a water-filled bucket, and agitated for uniform sample distribution. The sieve is lifted out of the bucket and one-half of the sample deposited into a sample jar, one-half into another. Depending on particular QA/QC objectives, each jar is labeled accordingly.

7.1.1 QA/QC

Macroinvertebrate identification is done by either contracted laboratories or in-house staff. However, regardless of where the identification takes place, a QA/QC component of identification is required. The mandatory, random selection and re-processing of at least 10% of all samples should conform to the following rules:

Sorting: At least 10% of the samples shall be re-sorted and for each sample designated for re-sorting:

- 1) At least 95% of the organisms have been removed for counting and identification from the sample; and
- 2) At least 95% of the taxa are contained within the original sort.

Identification and Counting: At least 10% of the sorted samples shall be re-identified and re-counted by a separate taxonomist and for each sample designated for re-identification and recounting:

- 1) At least 95% of specimens are correctly identified and;
- 2) At least 95% of the specimens are correctly counted.

If these criteria are not met, then the sample has to be re-processed. If the criteria cannot be met after three attempts, then a letter must be submitted to the Department stating that the QA/QC protocol could not be met and a brief statement for the reasons why it could not be met (i.e. limited taxa diversity). The results of QA/QC samples should be provided to NHDES in a separate MsExcel table.

7.1.2 Sorting and Subsampling

Over the years the program has used two methods of subsampling. One method, developed by Maine Department of Environmental Protection, requires all organisms to be

sorted from the detritus first, after which they are suspended in an aqueous solution in an Imhoff cone. The other method, the Caton Grid method, uses a meshed screen to evenly distribute the sample, which is then divided into “cookies”. This method, explained in the EPA’s Rapid Bioassessment Protocols, 2nd Ed, is the current method for the program. The Imhoff cone method is described briefly below only to aid in understanding historical data processing, from 1997-98.

Maine DEP Sub-sampling Method

After sorting, the macroinvertebrate sample is placed into an Imhoff-type settling cone and water is added to bring the total volume to one liter. The sample is then agitated with an aquarium air stone sealed in the bottom of the cone and connected to a compressed air supply. Large or dense organisms, such as crayfish or some Trichoptera, should be removed from the material to be sub-sampled and counted separately.

After several minutes, 25% of the sample is removed in five aliquots with a wide mouth dipper and placed in a sample vial. At this point the sub-sampling technician should ascertain whether 100 organisms have been removed. If less than 100 organisms have been removed after the 25% sub-sample has been taken, then the entire sample should be identified.

EPA Caton Method

Subsampling performed following the EPA approved “Caton Method” involves the use of a standardized gridded screen which contains 30 uniform squares of 6 cm² each. The screen portion fits into a slightly larger tray so that water may be added to the sample. Once water is added, the sample is dispersed over the screen. Special care is taken to completely distribute the sample over all the grids; each grid has an equal probability of being selected. The screen is then removed from the tray, causing the organisms to settle on the screen (Figure 7-1).



Figure 7-1. Subsampling using the Caton Grid Method.

Biologists use a random numbers table to choose grid numbers for organism selection. Organisms adhering to the screen are sub-sampled using a 6 cm² metal square to delineate sample size. A special scoop is used to remove the organisms from the screen. Organisms that occupy more than one grid are considered to be in the grid that contains the head. For organisms with no discernable head, the organism is considered to be in the grid containing the largest portion of the organism. As with the Maine DEP method, 25% of the sample is randomly picked (7.5 6 cm² squares) with a 100-organism minimum. If 100 organisms are not present in the subsample, then the entire sample must be sorted and identified.

7.2 Contracted Taxonomy



Samples are logged in at the time of receipt and checked against the enclosed packing list. DES is contacted if missing, damaged, or anomalous samples are noted. Shipment receiving date and initials of check-in personnel are entered on the contract Laboratory Tracking Sheet (LTS). Internal and external sample labels are checked for consistency and the department contacted if discrepancies are noted.

Sample processing will be initialed on the lab tracking sheet at the completion of each step by the individual having custody and doing the actual work. Sub-sampling will comprise 25% of the total sample. The Lab Tracking Sheet, voucher collection (see voucher section), and the remainder of the samples are returned to DES upon completion.

The contractor will be responsible for all equipment, facilities, materials and personnel with the exception of supplying the original sample containers necessary to collect and ship the department's macroinvertebrate samples to the contractor.

All samples, voucher specimens, electronic and paper data, and tracking QA/QC data sheets will be provided to the department in the prescribed format and considered to be department property as the laboratory processing for a sample lot is completed by the contractor.

The contractor is responsible for adhering to all federal, state, and local laws pertaining to the proper return shipment (including packaging) and storage and disposal of chemicals and/or other waste products generated in the processing of department samples.

7.3 In-House Identification

An estimate of the number of macroinvertebrates per sample determines the need for sub-sampling and sorting. In-house subsampling is done by the EPA Caton Method. Documentation of identification is performed on the **NHDES Biomonitoring Program Macroinvertebrate Identification Log sheet** (Appendix A-5). Identification level is determined by the purposes of the study project and should be to the lowest taxonomic level possible. Identified samples are to be retained in ethanol filled containers that are sorted by family. These vials shall be labeled by site name or station ID, replicate number, and complete taxonomic information.

8. Data Entry

Data recording and transcription from field sheets to the database is monitored and checked for accuracy. Data sheets discussed in previous sections include: **Electrofishing Data Sheet**, **Habitat Assessment Field Data Sheet**, **Stream Flow Measurements Data Sheet**, and the **Biomonitoring Site Information Sheet**. The information recorded on these sheets is transferred to the Ecological Data Assessment System (EDAS).

8.1 EDAS

The EDAS database was recommended by the EPA and the Biomonitoring Program has customized the original version (V3.0) to best meet the program needs. The database

manages chemical, physical, and biological data using a Microsoft Access platform. Entry of data into EDAS is performed through a data entry switchboard, allowing for the selection of one of several subforms into which data is actually entered.

The switchboard contains the following subforms: Station Information, Hydrolab Data, Fish Data, Habitat Assessments, Macroinvertebrate Data, and Flow Data. Sample pages of the switchboard and each subform, explanations of the fields contained in each, and a diagram of the relationships among fields within a form and among forms are found in Appendix B.

Once in a form, click on the **New Station** icon in the top left corner. Enter new data into appropriate fields.



Additional features are also available in the subforms, including **Find** (little binoculars) and **Master Update**. The **Find** function allows for data searches based on any of the fields in the subform. The **Master Update** function allows for data to be changed and also prevents the user from accidentally deleting or altering data. It requires a password to change already existing data.



At this time there is also a few select queries located on the switchboard. They are Station Summary, Fishes of New Hampshire, Total Fish, and Total Habitat Scores. . The switchboard is constantly changing, and more queries and reporting tasks will be added as needed.

8.2 ARC VIEW/GIS

The Biomonitoring Program collects Latitude/Longitude information at every site. These are recorded on the field data sheets, and in the Stations EDAS form. However, in order to produce maps in ArcView, the Lat/Long data is given in table format to the NHDES GIS group. They update the biomonitoring station coverage. All sites from 1997 to present are in a data layer.

In addition to a "point" coverage, the program also has a polygon data layer that shows the unique watersheds of each of the stations. This data is created by the GIS specialists also. At the end of each season, the program will have an updated point coverage and an additional coverage showing all the microwatersheds for each station. Figure 8-1 shows the current stations and watershed coverages.

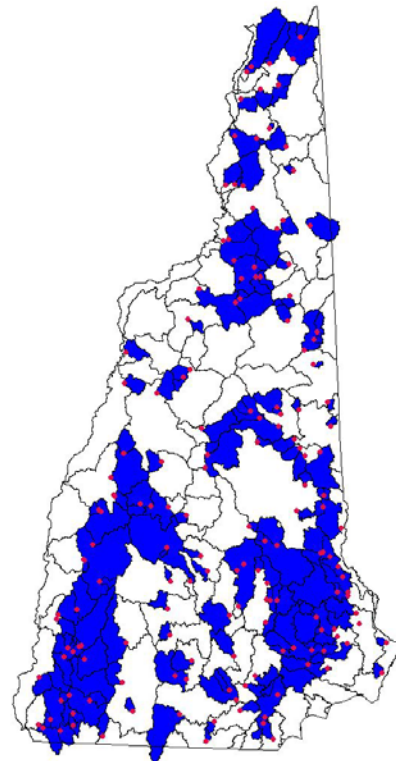


Fig 8-1. Biomonitoring Stations and Microwatersheds, 1997-2001



8.3 QA/QC of Data

Quality assurance and quality control of previously mentioned data types is an integral part of the Biomonitoring Program and will be performed on all data for all projects. After entire data sets for specific projects have been entered into EDAS, review of entered data will be performed, optimally by someone other than the person who entered the data. The tabular, non-reviewed raw data from EDAS will be compared to field data sheets for correctness and the raw data sheets will be initialed by the reviewer. Errors such as duplications, omissions, erroneous results, etc... are reported to the program coordinator, who will then make the necessary changes. Additional QA/QC records will be retained with the project raw data folders.

9. Voucher Collection

9.1 Macroinvertebrates

The contract laboratory will produce a reference collection that supports all the macroinvertebrate identifications within a particular survey. Voucher specimens will be placed in vials with polyseal caps and immersed in the preservation fluid (ethanol). Internal labels will contain relevant taxonomic information (i.e. Order, Family, genus, species), station ID, site name, and collection date. Multiple specimens from a single taxon and from a single original sample can be housed within the same vial. Specimens should not be mixed between stations or replicates from a site.

Specimens necessitating mounting on microscope slides (i.e. midges) shall be done so utilizing non-water based mounting media. Specimens will be mounted on 3"X 1" inch slides and labeled on the left hand side so that the mounting media is known as well as the specimen collection location and date. Space will be retained on the right side of the slide for a voucher collection specimen number.

9.2 Fish

The Biomonitoring Program does not as yet have a voucher collection for fish species. This is in part due to lack of storage space that would be required. At this time the procedure is to retain representative specimens only from taxa that are unclear or in question. These are put in ethanol for later verification. The program is exploring options for voucher collections of fish, including the possibility of a photo voucher of all taxa from each site.



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